IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	DAUNER, Michael; SCHAUB, Jochen
Serial No.:	10/598,377
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Title:	METHOD AND DEVICE FOR THERMAL CONDITIONING OF A CELL
Group:	1657
Examiner:	LILLING, Herbert J.
Attorney Ref.:	PAT 62246W-2 US

Declaration under 37 C.F.R. § 1.132

I, Joachim Schmid, of Schwenninger Str. 10, 71069 Sindelfingen, Germany, declare and state as follows:

1. I am employed at Insilico Biotechnology AG, the assignee of the invention that is the subject of the above-noted application to which this declaration pertains.

2. I have been involved for 9 years in research and development relating to biotechnology, more specifically, cell cultures and modeling thereof. I graduated with a degree in Process Engineering in 2000 and received a Ph.D. in Engineering Sciences 2007.

3. In order to recover metabolites from cells, a number of methods have been employed in the prior art. These methods generally involve the lysis of the cells, in which the cell membrane is destroyed and the component materials are released. The cell debris, naked DNA, and protein complexes are present in the cell suspension along with the desired liberated metabolites. This means that purification steps must be performed. The present inventions seeks to provide a method in which metabolites are recovered

with minimal cell damage, resulting in less contamination of the metabolites with interfering materials, such as cellular debris and protein complexes.

4. Lee et al. (US 6,197,553) (henceforth Lee) discloses the thermolysis of E. coli cells, i.e the destruction of biological cells by thermal means. Lee accomplishes this by suspending microbial cells in buffer and heating the suspension to about 70-100°C in a flow-through heat exchanger. As disclosed in Lee (see Abstract), the thermal treatment lyses the cells, and is meant to replace other techniques commonly used in the art to accomplish this, such as alkaline lysis, lysozyme supported lysis (enzymatic digestion), and isopycnic centrifugation (see the "Background of the Invention" in columns 1 and 2 of Lee). These treatments are commonly known to result in complete lysis of the cells subjected thereto.

5. This is in contrast to the present invention, which expressly seeks to avoid full lysis of the cells, but rather seeks to obtain the contents from the cell, i.e. the metabolites, while avoiding contamination of the samples by the cell debris, which would be produced by full lysis. To achieve this goal, the present invention requires that the cells be subject to a particular thermal conditioning step, characterized by a heat transfer to the cells that is best characterized by a "thermal equivalent" (WE) of 90 to 150 K·sec at a conditioning temperature T_K of 80 to 95°C, in which $WE = t_h(T_K-T_M)$ (t_h is the conditioning time, T_K is the conditioning temperature, and T_M is the temperature of the cell medium before entering the conditioning treatment).

6. In order to demonstrate that Lee differs from the present invention, I have examined Lee's examples and calculated the thermal equivalents used by Lee in each of his

relevant examples, and determined that these fall outside the scope of the present invention.

7. According to Example 1 of Lee, a cell suspension (starting temperature of 24°C) is pumped through a capillary of a heat exchanger (bath temperature 92°C) at a flow rate of 81 mL/min which results in a residence time within the capillary of about 35 seconds. The inlet and outlet temperatures of the cell solution were measured to be about 24°C and 89°C, respectively. In Example 2, the bath temperature was set to 96°C and the flow rate was increased (in comparison to Example 1) to 160 to 850 mL/min, resulting in a shorter residence time. Accordingly, the outlet temperatures ranged between 93°C and 65°C, corresponding to a residence time of 17.7 second to 3.3 seconds, respectively. In example 5, the bath temperature was set to 100°C and the flow rate was set to 500 mL/min with a residence time of 5. 7 seconds and a corresponding outlet temperature between 70°C-77°C. I calculated the residence times for Example 2 as follows:

For a flow rate of 850 mL/min:

$$81 \text{ mL/min} / 850 \text{ mL/min} \cdot 35 \text{ sec} = 3.3 \text{ sec};$$

for a flow rate of 160 mL/min:

$$81 \text{ mL/min} / 160 \text{ mL/min} \cdot 35 \text{ sec} = 17.7 \text{ sec}$$

I further calculated additional values inbetween the values given in the text. These additional values were taken from figure 2 of Lee:

For a flow rate of 405 mL/min (taken from figure 2):

$$81 \text{ mL/min} / 402 \text{ mL/min} \cdot 35 \text{ sec} = 7.0 \text{ sec};$$

for a flow rate of 320 mL/min (taken from figure 2):

81 mL/min / 320 mL/min · 35 sec = 8.9 sec

For Example 5, I calculated the following residence time:

For a flow rate of 500 mL/min:

81 mL/min / 500 mL/min · 35 sec = 5. 7 sec.

Examples 3 and 4 describe lyses that utilize conditions described in Examples 1 and 2.

Example 5 additionally includes the utilization of lysozyme, which results in a complete lysis of the cells and thus the occurence of impurities (see table 1 of Lee) which the invention under examination seeks to avoid.

8. To calculate the "thermal equivalent" (WE), from the above values, the actual momentary average temperature within the capillary of the heat exchanger is calculated and integrated over time. The equations shown below are derived from basic thermal dynamics and fluid mechanics. The following boundary conditions are applied: there is a constant wall temperature at the capillary; a thermally and mechanically fully developed laminar flow exists within the capillary. Subject to these boundary conditions, the average momentary temperature (bulk temperature) T_b and the wall temperature T_w can be calculated from the energy balance, which yields

$$\frac{T_w - T_b}{T_w - T_{b1}} = e^{-Cx} = e^{-ct}$$

where T_{b1} is the inlet temperature, T_w is the wall temperature, x is the position (distance from the inlet), and C is a constant value that depends on material

characteristics and capillary geometry. Since position x and residence time t are proportional owing to a constant mean velocity (directly following from mass conservation), c is also a constant value that is derived from C. For the temperature T_{b2} at the outlet position x=b (residence time s), it thus follows:

$$T_{b2} = T_w + (T_{b1} - T_w)\, e^{-Cb} = T_w + (T_{b1} - T_w)\, e^{-cs}$$

$$T_{b2} = T_{b1} + (T_w - T_{b1})\,(1 - e^{-cs}) \Rightarrow c = -\frac{1}{s}\ln\left(1 - \frac{T_{b2} - T_{b1}}{T_w - T_{b1}}\right)$$

$$T_b = T_{b1} + (T_w - T_{b1})\,(1 - e^{-ct})$$

$$\int T\,dt = \int (T_w - T_{b1})\,(1 - e^{-ct})\,dt$$

$$\int T\,dt = -\frac{1}{c}(T_{b1} - T_w)\,[ct + e^{-ct}] = -\frac{1}{c}(T_{b1} - T_w)\,(cs + e^{-cs} - 1)$$

$\int T\,dt$ corresponds to "thermal equivalent" (WE) in K·s

9. Using the above equations, the following values were calculated for Examples 1 and 2 of Lee:

example of Lee *et al.*	residence time	outlet temperature	$\int T\,dt$ = WE
1	35 sec	89°C	1651 Ks
2-a	17.7 sec	93°C	890 Ks
2-b (interpolated)	approx. 8.9 sec	approx. 85°C	approx. 352 Ks

2-c (interpolated)	approx. 7.0 sec	approx. 80°C	approx. 243 Ks
2-d	3.3 sec	65°C	77 Ks

Example 5 uses lysozyme and is therefore not considered further in this comparison.

10. Claim 1 of the present application requires that WE be in the range of 90-150 K·s at a conditioning temperature of 80 to 95°C. I note that Lee uses conditions requiring a WE far outside the range of the present invention. Even at a outlet temperature as low as 80°C (example 2-c) the calculated WE amounts to 243 Ks which is outside the range of claim 1. At lower outlet temperatures, especially of 65°C in example 2-d, the conditions are outside the range of claim 1, which further requires the conditioning temperature to be from 80 to 95°C.

11. This is in keeping with the purpose of Lee versus the purpose of the present invention. The present invention, while using thermal means to extract the metabolites, does not actually lyse the cells as required in Lee's teaching. Lee's example 1 describes an effective cell lysis by the thermal treatment (column 6, lines 61-62). Lee's example 2 is silent about cell lysis, example 2 rather concerns the yields of circular DNA from cell supernatant (column 7, lines 26-28). . However, Lee asserts that complete cell lysis is required to yield DNA therefrom (e.g. column 2, lines 49-67). Lee does not mention a condition where cells readily release their contents (metabolites) without occurrence of cell lysis (as achieved by the invention under examination). Lee did not discover nor disclose that cell contents could be obtained without full lysis of the cells.

12. In my opinion, the above calculations and arguments illustrate and support the distinctions between the subject matter claimed in the present application and Lee. The time and temperatures used by Lee differ from the requirements of the presently claimed invention.

13. I hereby declare that all statement made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that

these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Date: 3 March 2009

Joachim Schmid

Joachim Schmid